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ORIGINAL PAPER

Impact of harvest residues, fertilisers and N-fixing plants on growth and nutritional status of young *Eucalyptus globulus* plantations, under Mediterranean conditions

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Abstract Growth and nutritional status of young plants of *Eucalyptus* were assessed in a field trial, under different scenarios of harvest residue management and nutrient availability. Treatments were as follows: incorporation of harvest residues into the soil by harrowing (I); I with N fertiliser application (IF); I with leguminous, *Lupinus luteus* L., seeding (IL); removal of harvest residues (R); R with N fertiliser application (RF); R with leguminous seeding (RL); distribution of harvest residues on the soil surface (S); S with N fertiliser application (SF). Treatments were replicated four times in four blocks with a fully randomised design. Tree growth (height and diameter at breast height) was measured and understory biomass destructively recorded. Tree nutritional status was assessed by foliar analysis (N, Ca, Mg, P, K and leaf area). Significant differences in growth between I, R and S treatments were only detected at early stage. Intercropping with *Lupinus* decreased tree growth during the early phase, but after 5 years growth was similar to that measured in the I and R treatments. Application of fertiliser enhanced tree growth especially when harvest residues were retained on the soil surface. Combining incorporation of harvest residues with fertiliser application (IF) was the best option to increase tree growth, which was significantly greater than in the R and S. Initially, leaf N was positively affected by the leguminous (RL and IL), but, after the first fertiliser application (1 year after planting), greater N was observed in the IF, RF and SF, the difference decreasing gradually over the following years.

Keywords Tree growth · Soil · Legumes · Nutrition status · SPAD · *Eucalyptus*

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Introduction

Eucalypt plantations, intensively exploited as coppice stands, cover approximately 1.3 million ha in the Iberian Peninsula, 54% of which are located in Portugal (MMAMRM 2006). Removal of harvest residues has been frequently practised to facilitate access and soil preparation for the following rotation. As these residues contain a large amount of nutrients (Cortez 1996; Spangenberg et al. 1996; Jones et al. 1999), their removal may result in depletion of organic matter and nutrients and, consequently, in decreased soil fertility. In fact, a decrease in the productivity has been observed in second and further rotations of *Eucalyptus* (Merino et al. 2003) and other tree species plantations (Smethurst and Nambiar 1990; Proe and Dutch 1994; Khanna 1997). However, in Portugal, under sub-humid Mediterranean conditions, the decline in productivity resulting from removal of aboveground organic residues (litter and harvest residues), either in replanted or coppiced plantations, has not been yet observed in eucalypt stands (Jones et al. 1999; Madeira et al. 2004), as also reported in experiments for other Mediterranean climate conditions (e.g. Australia; Mendham et al. 2003).

Appropriate management of harvest residues and nutrient cycling has been considered necessary to enhance site productivity in short-rotation eucalypt plantations (Adams and Attiwill 1986; Shammas et al. 2003). Nowadays, the removal or maintenance of harvest residues (either on soil

surface or incorporated into the top layers) is strongly debated, not only due to the relationship between harvest residue management and improvement of soil quality, but also drive by the possible use of harvest residues as source for energy production to partially replace fossil fuels (Cowie et al. 2006; Stupak et al. 2007).

Fertiliser application has been reported as a management practice which often improves tree growth (Gonçalves et al., 1997; Fisher and Binkley, 2000). Although water availability is the main constrain to forest productivity under Mediterranean conditions (Portugal), the availability of N and P have been also considered as limited factors (Pereira et al. 1996). However, application of fertilisers affected eucalypt production at low extent (Pereira et al. 1989; Madeira and Pereira 1990/91) in the first rotation, but these studies were carried out in the absence of organic layers and harvest residues. Previous studies in Portugal on the management of harvest residues did not include the application of fertilisers (Jones et al. 1999; Madeira et al. 2004), so that the potential growth associated with the interaction between residue management and fertiliser application is unknown. Therefore, it is important to understand, in Mediterranean conditions, the combined effect of different systems of residue management and use of fertilisers or N-fixing plants on the productivity of eucalypt plantations.

In this context, an experiment was established in a representative (climate, geology and soils) area of eucalypt plantations in Central Portugal to: (1) evaluate the influence of harvest residue management (removed, distributed on the surface or incorporated into the soil) and the use of N fertilisation and intercropping N-fixing leguminous plants on tree growth and nutritional status, to identify the most appropriate management system to increase eucalypt plantation productivity; (2) assess interactions between harvest residue management, fertiliser application and N-fixing leguminous plants.

Materials and methods

Site characteristics

The study was carried out in an area of western-central Portugal (Quinta do Furadouro, Óbidos, lat. 39°21′N, long. 9°14′W, altitude 50 m), about 10 km from the Atlantic Ocean. The geologic formations belong to the Cretacic (sandstones of the formation "Grés de Torres Vedras"; Zbyszewski and Almeida 1960), and the landscape is flat to gently undulating, with slopes from 5 to 12%. Soils are predominantly Haplic Cambisols (Dystric) and Haplic Regosols (Dystric) (WRB 2006), with coarse fragment content less than 60 g kg^{-1} and loam to clay loam texture, pH (H_2O) values lower than 5.3, small organic C contents (10.9–24.3 g kg^{-1}) and small extractable P contents (<5 mg kg^{-1}) (Jones et al. 1999). Soils do not show compact layers and rooting depth limit is beyond 1 m. The natural vegetation belongs to the series *Asparago aphylli-Querceto suberis sigmetum* (Costa et al. 2002), dominated by *Quercus faginea* subsp. *broteroi* (Cout.) A. Camus and *Quercus suber* L. Some other tree and shrub species which also occur in the area include *Pinus pinaster* Ait., *Pinus pinea* L., *Phillyrea latifolia* L., *Quercus coccifera* L. and *Castanea sativa* Mill.

The climate of the region is Mediterranean, with a relative humidity above 80% throughout the year due to oceanic influence, which partially contributes to offsetting summer drought. As recorded by the nearest meteorological station (Caldas da Rainha, lat. 39°24′N, long. 09°08′W, alt. 70 m), the 30-year mean total rainfall is 607 mm and mean annual air temperature is 15.2°C, ranging from a monthly mean of 10.4°C in January to 19.8°C in August (Reis and Gonçalves 1981). During the experiment, 2004 and 2005 were the driest years (501 and 456 mm, respectively); the wettest year was 2006 (1,043 mm) followed by 2003 (810 mm) and 2002 (765 mm) (INAG 2009).

Treatments and experimental design

The experiment was installed in a replanted area of *Eucalyptus globulus*, after the harvest (Autumn 2001) of the previous 34-year-old plantation, which was coppiced twice before. Stump sprouting was controlled by glyphosate (Roundup®) application, to prepare the area for plantation of eucalypt seedlings between stump rows. The experiment was set up in March 2002 and consisted of eight treatments laid out in four blocks in a fully randomised design. The treatments were as follows: incorporation of not crushed harvest residues in topsoil by harrowing to 20 cm (I); I with subsequent N fertilisation (IF); I with *Lupinus luteus* L. (IL); removal of harvest residues from soil surface (R); R with subsequent N fertilisation (RF); R with *Lupinus* (RL); distribution of harvest residues on soil surface (S); S including subsequent N fertilisation (SF). The forest floor litter was kept in all treatments.

In each treatment plot, an area of 225 m^2, corresponding to 25 trees, was surrounded by two rows of trees, giving a total plot area of 729 m^2. Harvest residues in the R, RF and RL treatment plots were manually removed from the site; in the plots S and SF residues were uniformly distributed by hand on soil surface, and incorporated into the soil by a disc harrow (14 t, with 80 cm diameter discs) in the I, IL and IF plots. The leguminous plant was sown in the RL and IL plots by scarification, and 110 kg of seed and 220 kg of superphosphate at 18% were applied per ha, as advisable for N-fixing plants (David Crespo, personal communication).

The three-month old *E. globulus* seedlings (control crossed seedlings, produced by Celbi SA) were planted by hand in March 2002, after treatment application, at 3×3 m^2 spacing. Each seedling was then supplied with 150 g of a NPK commercial fertiliser (12% N-NH_4/N-NO_3, 24% P_2O_5 and 12% K_2O), half of the amount being distributed in each side (at 20–25 cm) of the plant.

One, two and three years after planting (in March 2003, 2004 and 2005, respectively), a N-based fertiliser (ENTEC 26- 7.5% NO_3, 18.5% NH_4, 32.5% SO_3) was manually applied (200 kg/ha) on the treatment plots SF, RF and IF. Only N was applied in this experiment as a previous study showed that N was the most limiting factor for *E. globulus* growth (Azevedo et al. 2004).

Measurements and sampling

Soils were described based on six profiles. Sampling for chemical characterisation of soils was done with an auger at 0–10, 10–20, 20–30, 30–50 and 50–70 cm depths, in the centre and in each corner of each plot (totalising five samples per plot), and subsequently combined with a composite plot sample. Forest litter and harvest residues (divided into leaves, barks, twigs, branches and fruits) resulting from the previous *E. globulus* plantation were quantified by randomly sampling 1 m^2 areas, in a total of 15 replicates. All residues were then dried at 80°C and weighed.

The height of all trees per plot was measured at 7, 18, 29, 42, 53 and 68 months after planting (MAP). The diameter at breast height was measured at 29, 42, 53 and 68 MAP. As only small shrubs occurred, a wooden frame measuring 0.5×0.5 m^2 was randomly applied four times in each treatment plot, every spring from 2002 to 2006 for aboveground biomass sampling. All the understory plants inside the frame were harvested by clipping vegetation close to the ground and separately packed in identified plastic bags. They were immediately oven-dried in the laboratory at 80–85°C, and weighed.

Nutritional status of the trees was assessed on the basis of leaf growth analysis and nutrient contents. Eight leaves of the season's growth were sampled, from the upper third of the unshaded crown of two trees randomly chosen per treatment and block, at seven sampling dates. In the first two sampling dates (at 8 and 14 MAP), leaves were mainly juvenile, not fully expanded; however, at 20 and 26 MAP, and varying with the treatment, a mixture of juvenile expanded leaves and adult leaves was sampled. From 32 MAP onwards, only adult leaves were sampled.

Methods of analysis

Tree nutritional status was assessed by foliar chemical analysis. Leaf growth measurements included area (*Portable Area Meter Model Li-3000A, LI-COR*), and fresh and dry weights. Specific leaf area (SLA, cm^2 g^{-1}) was calculated as the mean ratio between the areas and dry weights of eight leaves.

Leaves were oven-dried (80°C for 48 h) to a constant weight and a sub-sample was milled (1 mm sieve) for chemical analysis. Leaf N content was determined by Kjeldahl digestion analysis (*Digestion System 40, Kjeltec Auto 1030 Analyser*). The solubilisation of the residue elements (P, Ca, K and Mg) was obtained by digestion (*CEM Microwave Digestion System Model MDS-81 D*), using 0.5 g of material in 10 mL HNO_3 at 65% (LDV tubes). The resulting solution was evaporated in "Fourneau" glasses, the respective residue being solubilised by the addition of 10 mL HCl 3 M. Subsequently, Ca, K and Mg were determined by atomic absorption spectrometry (AAS), and P was quantified by the ascorbic acid method.

Soil pH was determined by the potentiometric method in water (soil/solution ratio 1:2.5). Organic C was estimated by wet oxidation and total N as previously described. Extractable P was determined by the Egnér-Riehm method, exchange base cations were extracted by NH_4OAc 1 M, adjusted to pH 7.0, and extractable Al was determined after extraction with KCl 1 M. Contents of Ca, Mg, Na, K and Al were determined by AAS and P by colorimetry.

Calculations and statistical analysis

The measured and estimated parameters were statistically compared between treatments, using the SPSS Statistical Program for Windows, version 13.0 (*SPSS Inc.*). The normality of variance was confirmed by the Kolmogorov–Smirnov test and the homogeneity verified by Levene test. Whenever the homogeneity was confirmed, the comparisons were performed through one-way anova procedure and Tukey test, considering treatments as independent variables and the parameters as dependent variables. When the homogeneity of variance was not confirmed, the averages were compared through Dunnett's T3 test. The significance level of 0.05 was used in all statistical analysis.

Results

Nutrients in harvest residues and forest litter

The amount of harvest residues (Table 1) at beginning of the study was about 38 t ha^{-1}, comprising mainly leaves (11 t ha^{-1}) and branches (18 t ha^{-1}), the amount for twigs and barks being 5 and 4 t ha^{-1}, respectively. Harvest residues contained a considerable amount of N and Ca, but P was much smaller than the other nutrients. Nitrogen (66%) and P (56%) occurred mainly in leaves, while Ca was

Table 1 Amounts of biomass (BM) and nutrients in harvest residues (HR) and forest litter (FL)

	BM (t ha^{-1})	N (kg ha^{-1})	Ca (kg ha^{-1})	Mg (kg ha^{-1})	K (kg ha^{-1})	Mn (kg ha^{-1})	P (kg ha^{-1})
HR	38.39	165.3	442.8	37.7	68.2	31.3	15.3
Leaves	10.98 (7.45)	109.9 (9.8)	160.6 (17.8)	14.7 (0.7)	23.8 (2.8)	13.1 (0.6)	8.6 (1.0)
Branches	17.76 (10.76)	31.2 (4.7)	139.4 (16.2)	11.9 (0.6)	25.6 (4.0)	10.3 (0.6)	3.8 (0.7)
Twigs	5.09 (1.35)	14.7 (0.8)	70.9 (3.8)	5.2 (0.2)	14.0 (0.8)	3.9 (0.1)	1.8 (0.1)
Barks	4.26 (4.49)	8.4 (0.8)	70.1 (15.4)	5.6 (1.1)	3.8 (1.0)	3.9 (1.0)	0.9 (0.3)
Others	0.31 (0.26)	1.2 (0.1)	1.8 (0.1)	0.3 (0.0)	1.0 (0.2)	0.1 (0.09)	0.1 (0.0)
FL	20.6	174.4	412.0	39.2	37.0	ND	13.0

Values are mean and standard deviation (between brackets)

ND not determined

distributed between leaves and branches. Except for K, nutrient amounts in harvest residues were similar to those measured in forest litter.

Soil characteristics

Soil organic C and N contents (Table 2) were low, and the C/N ratio was high especially in the 0–10 cm soil layer (25.9). The contents of exchangeable base cations pointed to their reasonable availability. The extractable Al was negligible in the 0–10 cm (0.15 $cmol_c$ kg^{-1}), but increased with depth. The extractable P was low (about 6 mg kg^{-1}) down to 30 cm, but the content down to 20 cm was equivalent to that observed in the harvest residues at beginning of the study. In contrast, extractable K (62–92 mg kg^{-1}) was sufficient to supplement the needs of trees.

Tree growth

At the end of the study, at 68 MAP, trees in the I treatment were taller than in the S and R treatments (Table 3). Tree height was 13.6, 12.2 and 12.1 m, respectively, and diameter at breast height (DBH) reached 10.7 cm in the former and 9.1 and 9.0 cm, respectively, in the R and S treatments. However, differences between treatments were not significant.

Although there were early symptoms of tree growth depression associated with the intercropped *L. luteus*, significant effects were only observed at 7 MAP, when tree height in the IL (1.0 m) was significantly shorter than in the treatment I (1.7 m). At the end of measurements (at 68 MAP), the IL trees were still shorter but closer to those in the I (13.2 and 13.6 m, respectively), while were taller in the RL (13.8 m) than in the R (13.4 m). A similar pattern was observed for DBH values (Table 3).

The N-based fertiliser, applied 1 and 2 years after planting increased the height of trees in the IF (8.0 m), SF (7.1 m) and RF (6.0 m), differences being significant between SF and S, and between IF and IL (at 29 MAP; Table 3). Trees in the IF, SF and RF were 1.27, 1.48 and 1.23 times taller than in the respective non-fertilised I, S and R; DBH values were 1.33, 1.51 and 1.31 times larger, respectively. After the third fertiliser application, height differences between fertilised and non-fertilised trees became not significant.

However, tree height in the IF was statistically taller than those measured in the R and S treatments (Table 3). The effect of residue removal (R treatment) may have been offset by fertiliser application as growth in the RF was similar to that observed in the I and S treatments.

Understory biomass

Understory biomass showed a strong variation between treatments during the early phase of the experiment (Table 4). At the end of the first spring after planting (before the first fertiliser application), understory biomass was much greater in the RL and IL treatments (356 and 250 g m^{-2}, respectively), than in the others (31–170 g m^{-2}); biomass in the I and IF (33 and 31 g m^{-2}, respectively) was smaller than in the S and SF (139–170 g m^{-2}), and R and RF (167 g m^{-2}), and significantly lower than in the RL and IL. After the early experimental stage, differences between treatments strongly decreased.

Foliar nutritional status

Changes in leaf nutrient contents (N, P, Ca, Mg and K) and N/P ratio are shown in Table 5. No significant differences were observed between treatments I, S and R during the study period.

Before fertiliser application, at 8 MAP, leaf N was greater in the IL (26.7 mg g^{-1}) and RL (25.5 mg g^{-1}) than in the other treatments (21.0–21.8 mg g^{-1}). However, after 32 MAP, although leaf N was still greater in the IL and RL than in both non-fertilised (I, R) and fertilised (IF, RF) treatments, differences were not significant. Later, at 44

Table 2 Contents of organic C and N, exchangeable base cations, extractable Al (Al_e) and extractable P and K, and values for C/N ratio and pH (H_2O and KCl), down to 70 cm depth, at beginning of the study

Depth (cm)	C (g kg^{-1})	N (g kg^{-1})	C/N	Exchangeable base cations				Al_e (cmol$_c$ kg^{-1})	P (mg kg^{-1})	K (mg kg^{-1})	pH	
				Ca (cmol$_c$ kg^{-1})	Mg (cmol$_c$ kg^{-1})	K (cmol$_c$ kg^{-1})	Na (cmol$_c$ kg^{-1})				H_2O	KCl
0–10	13.6 (4.6)	0.53 (0.12)	25.9 (6.1)	2.96 (1.43)	0.66 (0.35)	0.27 (0.11)	0.08 (0.02)	0.15	6.2 (2.3)	91.9 (34.1)	5.0 (0.3)	4.1 (0.2)
10–20	6.8 (1.2)	0.36 (0.08)	19.5 (2.3)	0.75 (0.58)	0.49 (0.28)	0.17 (0.05)	0.05 (0.02)	0.62	5.9 (3.6)	70.3 (19.2)	5.0 (0.1)	4.0 (0.1)
20–30	5.4 (1.1)	0.24 (0.07)	23.0 (5.2)	0.75 (0.58)	0.49 (0.28)	0.17 (0.05)	0.05 (0.02)	0.81	6.3 (4.3)	61.8 (14.7)	5.0 (0.1)	4.2 (0.2)
30–50	3.2 (0.9)	0.24 (0.07)	13.3 (1.7)	0.62 (0.53)	0.67 (0.40)	0.17 (0.06)	0.07 (0.03)	–	2.8 (1.8)	64.2 (17.8)	5.3 (0.1)	4.4 (0.2)
50–70	2.7 (1.2)	0.21 (0.08)	13.0 (2.2)	1.34 (1.18)	0.99 (0.58)	0.16 (0.04)	0.17 (0.07)	–	1.6 (1.8)	51.1 (13.1)	5.0 (0.1)	4.1 (0.1)

Values are mean ($n = 32$) and standard deviation (between brackets)

and 50 MAP, significant differences were observed between the greatest N for the IL treatment and the R, SF and S smallest N contents.

Two months after the first fertiliser application (at 14 MAP), leaf N in the IF, RF and SF was statistically greater (22.4–23.6 mg g^{-1}) than in the others (14.7–16.5 mg g^{-1}). The N/P ratio was statistically higher (15.2) in the fertilised than in the non-fertilised treatments (11.7). In contrast, leaf Ca and Mg were significantly lower. Leaf N significantly decreased at 14 MAP, relatively to that measured at 8 and 20 MAP (Table 5), which may be associated with the surging of leaf discoloration and necrosis of juvenile foliage produced by the fungus *Micosphaerella* spp., which equally affected trees of all treatments.

The second N fertilisation applied to the IF, RF and SF did not equally affect these treatments at 26 MAP: leaf N was 28.3, 23.0 and 21.2 mg g^{-1}, respectively, IF and SF being statistically different. At 32 MAP, leaf N in the RF (13.9 mg g^{-1}) trees was statistically smaller than in the IL, R and RL (about 18.4 mg g^{-1}). Similarly, leaf P showed statistical differences between IL, R or RL (1.4 mg g^{-1}) and RF or SF (1.1 mg g^{-1}) treatments (Table 5). The third application of N fertiliser in the IF, RF and SF treatments seemed not to have significantly affected leaf N measured at 44 and 50 MAP.

The SLA (Table 5) was positively affected by *Lupinus* treatment, at 8 MAP, since a greater SLA was observed in the IL and RL treatments (186 and 180 cm^2 g^{-1}, respectively) than in the others (150–174 cm^2 g^{-1}), differences being significant in relation to treatments R and SF. The incorporation of residues into the soil (I) enhanced SLA (174 cm^2 g^{-1}) when compared with their removal (R, 164 cm^2 g^{-1}) or their placement on soil surface (S, 154 cm^2 g^{-1}), but differences were not significant. Fertiliser application did not significantly affect SLA (Table 5). Due to changes in leaf size related to development towards full expansion and maturity, SLA decreased with tree age and it was kept similar after 32 MAP, when only adult leaves were sampled.

Discussion

Nitrogen, Ca, P, Mg and K amounts in harvest residues were similar to those reported for *E. globulus* plantations in Portugal (Madeira et al. 2004) and those determined in an experimental area previously installed in an adjacent site (Jones et al. 1999). The amounts of P and Ca represented about 70 and 33%, respectively, of the extractable soil quantity down to 30 cm depth; N amount was 10% of the total to this depth. However, the maintenance of harvest residues on the soil surface did not enhance tree growth when compared with their removal, which is in agreement

Table 3 Tree height (m) and diameter at breast height (cm) in treatment plots during the study period

Date	Treatments*							
	I	IF	IL	R	RF	RL	S	SF
Tree height								
7 MAP**	1.7 (0.2)a	1.6 (0.2)a***	1.0 (0.1)b	1.3 (0.1)ab	1.2 (0.1)b***	1.0 (0.0)b	1.3 (0.3)ab	1.3 (0.2)ab***
18 MAP	4.3 (0.9)ab	5.2 (0.4)a	3.3 (0.4)bcd	3.1 (0.3)cd	3.3 (0.3)bcd	2.9 (0.5)d	3.0 (0.5)d	4.2 (0.4)abc
29 MAP	6.3 (1.2)ac	8.0 (0.3)bc	5.2 (0.4)a	4.9 (0.7)a	6.0 (0.7)ac	4.7 (0.5)a	4.8 (0.7)a	7.1 (0.8)c
42 MAP	8.4 (1.6)abc	10.2 (0.3)ac	7.6 (0.4)bc	6.9 (1.4)b	8.2 (1.0)abc	7.4 (0.4)bc	7.0 (1.2)b	9.3 (0.6)c
53 MAP	10.2 (1.5)ab	12.1 (0.1)a	9.7 (0.6)ab	8.7 (2.0)b	10.2 (1.2)ab	9.8 (0.4)ab	8.8 (1.6)b	11.2 (0.9)ab
68 MAP	13.6 (1.7)ab	15.2 (0.7)a	13.2 (0.6)ab	12.1 (2.1)b	13.4 (1.1)ab	13.8 (0.2)ab	12.2 (1.7)b	14.6 (1.1)ab
Diameter breast height								
29 MAP	4.5 (0.8)ac	6.0 (0.3)b	3.8 (0.5)a	3.5 (0.8)a	4.6 (0.6)ab	3.5 (0.3)a	3.7 (0.7)a	5.6 (0.7)bc
42 MAP	6.2 (1.4)ab	7.9 (0.1)a	5.7 (0.5)b	5.1 (1.3)b	6.3 (0.8)ab	5.6 (0.3)b	5.1 (1.1)b	7.2 (0.9)ab
53 MAP	8.0 (1.6)ab	9.8 (0.2)a	7.7 (0.5)ab	6.6 (1.7)b	7.9 (0.9)ab	7.8 (0.3) ab	6.6 (1.2)b	8.7 (0.8)ab
68 MAP	10.7 (1.5)ab	12.0 (0.5)a	10.7 (0.7)ab	9.0 (2.1)b	10.2 (0.8)ab	10.9 (0.3)ab	9.1 (1.5)b	11.0 (0.7)ab

Values are mean and standard deviation (between brackets). Different letters in the same line means significant differences ($P < 0.05$) between treatments by the Tukey test

* *I*-incorporation of harvest residues into the soil by harrowing, *IF*-as I and N fertiliser application, *IL*-as I and leguminous seeding, *R*-removal of harvest residues, *RF*-as R and N fertiliser application, *RL*-as R and leguminous seeding, *S*-distribution of harvest residues on the soil surface, *SF*-as S and N fertiliser application

***MAP* months after planting

*** Values before the first fertiliser application (March 2003)

Table 4 Understory biomass (g m^{-2}) at the end of spring during the study period

Year	Treatments*							
	I	IF	IL	R	RF	RL	S	SF
2002	33 (7)b	31 (13)b**	250 (30)a	167 (54)ab	167 (43)ab**	356 (57)a	170 (38)ab	139 (33)ab**
2003	320 (59)	440 (78)	449 (82)	352 (72)	423 (80)	465 (60)	344 (22)	384 (36)
2004	481 (55)	484 (31)	599 (203)	457 (82)	675 (107)	457 (52)	359 (59)	517 (59)
2005	416 (79)	524 (82)	599 (297)	265 (11)	544 (100)	294 (51)	356 (77)	515 (99)
2006	177 (26)	367 (106)	507 (144)	249 (35)	609 (149)	274 (14)	170 (12)	495 (152)

Values are mean ($n = 4$) and standard deviation (between brackets). Different letters in the same line means significant differences ($P < 0.05$) between treatments by the Tukey test

* See Table 3 for explanation of treatment codes

** Before the first fertiliser application (March 2003)

with results reported by Jones et al. (1999) for early growth of eucalypt plantations (in soils with similar characteristics of those of the present study) in central Portugal and northwest of Spain (Galicia), with different mean annual rainfall (605–650 and 1,051–1,187 mm, respectively). In contrast, our results do not support those obtained by Deleporte et al. (2008) in a sub-equatorial climate (Congo) and Merino et al. (2003) in rainy areas of Northwest Spain, where maintenance of harvest residues significantly improved the growth and nutritional status of trees. This may be attributable, on one hand, to Mediterranean conditions of the study area, in which tree productivity is mostly limited by water availability (Pereira et al. 1989, 1996) and, on the other hand, to inherently higher soil nutrient status than in the aforementioned studies. Our results may be also explained by the low proportion of nutrient amounts in harvest residues in comparison with those accumulated in organic layers and mineral soil layers (see Table 2), as for a given soil nutrient status, tree growth negatively correlates with the quantity of nutrients removed from the soil (Saint-André et al. 2008).

Despite negative effects of harrowing on soil organic C status (Madeira et al. 1989), the incorporation of harvest residues (which also includes the incorporation of forest floor litter) into the soil enhanced tree growth compared with its placement on the soil surface or removal. Differences

Table 5 Leaf concentration (mg g^{-1}) of N, P, K, Ca and Mg, N/P ratio and specific leaf area (SLA, cm^2 g^{-1}) for all treatments during the study period

	Treatments							
	I	IF	IL	R	RF	RL	S	SF
8 MAP (November 2002)								
N	21.8 (4.3)ab	24.5 (4.3)ab*	26.7 (3.4)a	21.2 (2.4)ab	21.0 (2.8)b*	25.5 (5.2)ab	21.8 (3.4)ab	23.3 (2.3)ab*
P	2.1 (0.3)a	2.1 (0.2)a	2.1 (0.2)a	2.2 (0.5)a	2.0 (0.4)a	2.1 (0.2)a	2.1 (0.3)a	2.0 (0.4)a
Ca	3.9 (0.8)a	3.8 (1.0)a	4.4 (0.9)a	3.9 (0.7)a	4.0 (0.5)a	4.4 (1.6)a	4.1 (1.0)a	4.0 (1.7)a
K	12.1 (2.6)a	12.5 (2.8)a	11.8(1.9)a	11.5 (1.1)a	12.0 (2.2)a	10.9 (1.3)a	10.8 (2.3)a	10.5 (2.8)a
Mg	1.4 (0.3)ab	1.2 (0.1)b	1.5 (0.1)a	1.4 (0.1)ab	1.5 (0.3)a	1.5 (0.2)a	1.6 (0.6)a	1.6 (0.6)a
N/P	10.4 (1.6)ab	11.6 (1.7)ab	13.0 (1.4)a	10.1 (1.3)b	10.5 (1.5)ab	12.4 (1.9)ab	11.0 (1.8)ab	11.9 (1.8)ab
SLA	174 (29)ab	169 (19)ab	186 (23)a	164 (12)b	158 (8)ab	180 (29)ab	154 (13)ab	150 (30)b
14 MAP (May 2003)								
N	16.5 (2.8)b	23.6 (2.2)a	16.2 (2.6)b	15.5 (2.0)b	23.5 (3.2)a	14.8 (2.0)b	14.7 (1.3)b	22.4 (2.8)a
P	1.4 (0.2)ab	1.7 (0.2)a	1.4 (0.2)ab	1.3 (0.2)b	1.5 (0.1)ab	1.3 (0.2)b	1.3 (0.2)b	1.5 (0.2)ab
Ca	8.4 (2.0)ab	5.7 (1.0)b	9.5 (3.0)a	9.2 (2.2)a	5.8 (1.2)b	8.8 (1.2)a	8.0 (1.6)ab	5.9 (1.1)b
K	9.3 (1.2)a	8.4 (1.2)ab	9.2 (1.0)a	8.0 (1.0)ab	8.0 (1.1)ab	7.4 (0.6)b	8.9 (0.7)ab	8.5 (1.5)ab
Mg	1.9 (0.3)a	1.4 (0.2)b	1.9 (0.4)a	1.8 (0.2)a	1.4 (0.2)b	1.9 (0.2)a	1.6 (0.2)ab	1.3 (0,1)b
N/P	11.7 (0.6)b	14.1 (0.7)a	11.6(0.6)b	11.7 (0.7)b	16.2 (1.6)a	11.8 (1.2)b	11.6 (0.8)b	15.3 (2.5)a
SLA	108 (10)a	117 (13)a	116 (11)a	108 (7)a	122 (23)a	105 (5)a	109 (8)a	112 (9)a
20 MAP (November 2003)								
N	25.8 (1.0)ab	28.5 (3.3)a	25.1 (2.5)ab	25.1 (2.1)ab	26.9 (4.2)a	26.4 (3.0)a	26.0 (2.3)a	27.5 (2.6)a
P	2.2 (0.2)a	2.5 (0.5)a	2.1 (0.3)a	2.1 (0.2)a	2.5 (0.3)a	2.2 (0.2)a	2.2 (0.2)a	2.4 (0.4)a
Ca	5.2 (1.1)abc	3.4 (0.5)d	5.2 (1.2)abc	5.3 (0.9)ab	3.4 (1.2)d	4.5 (0.9)bcd	6.4 (1.0)a	3.7 (0.8)cd
K	11.7 (1.7)a	12.3 (1.9)a	12.3 (1.2)a	11.3 (1.4)a	11.9 (1.4)a	12.3 (1.9)a	12.3 (1.6)a	12.6 (1.9)a
Mg	1.8 (0.4)a	1.5 (0.2)a	1.6 (0.2)a	1.7 (0.3)a	1.6 (0.2)a	1.7 (0.2)a	1.6 (0.2)a	1.7 (0.2)a
N/P	11.7 (1.0)a	11.9 (1.3)a	11.6 (1.0)a	12.1 (1.2)a	10.8 (1.0)a	12.0 (0.7)a	11.7 (0.5)a	11.9 (1.4)a
SLA	139 (13)a	129 (34)a	143 (36)a	124 (34)a	139 (21)a	150 (20)a	151 (20)a	134 (34)a
26 MAP (May 2004)								
N	23.4 (4.7)ab	28.3 (4.7)a	22.4 (4.4)ab	21.7 (3.1)b	23.0 (2.1)ab	21.6 (5.5)b	19.9 (3.8)b	21.5 (5.7)b
P	1.7 (0.3)ab	1.8 (0.3)a	1.6 (0.2)ab	1.6 (0.2)ab	1.6 (0.1)ab	1.4 (0.1)b	1.4 (0.3)b	1.6 (0.2)ab
Ca	10.7 (1.7)a	11.1 (2.1)a	9.5 (2.3)a	9.5 (1.1)a	9.1 (1.1)a	8.6 (1.7)a	9.0 (1.5)a	9.4 (1.7)a
K	8.8 (1.3)a	8.0 (0.8)a	9.0 (1.6)a	9.8 (1.6)a	8.6 (1.5)a	9.0 (1.0)a	8.8 (1.1)a	8.3 (1.1)a
Mg	2.1 (0.3)a	2.0 (0.3)a	1.9 (0.4)a	1.9 (0.3)a	1.7 (0.2)a	1.9 (0.4)a	1.8 (0.2)a	1.8 (0.2)a
N/P	14.1 (1.0)a	15.8 (2.7)a	14.3 (1.6)a	13.2 (1.0)a	14.7 (1.4)a	15.0 (2.8)a	14.0 (1.7)a	13.2 (2.5)a
SLA	107 (11)a	112 (18)a	108 (15)a	110 (16)a	104 (13)a	102 (13)a	101 (13)a	99 (5)a
32 MAP (November 2004)								
N	15.9 (1.7)ab	16.0 (1.7)ab	18.4 (2.6)a	18.2 (2.0)a	13.9 (1.0)b	18.4 (1.9)a	16.4 (4.3)a	14.6 (2.2)ab
P	1.2 (0.1)ab	1.2 (0.1)ab	1.4 (0.2)a	1.4 (0.2)a	1.1 (0.0)b	1.4 (0.2)a	1.3 (0.3)ab	1.1 (0.2)b
Ca	7.8 (1.3)a	7.6 (2.1)a	6.9 (1.1)a	6.1 (0.9)a	7.0 (1.1)a	5.7 (1.1)a	6.8 (1.6)a	7.6 (1.8)a
K	6.8 (1.2)ab	7.4 (1.3)ab	7.9 (1.3)ab	8.6 (1.5)a	6.0 (0.6)b	7.3 (1.2)ab	6.9 (1.7)ab	6.4 (0.9)ab
Mg	2.1 (0.4)a	1.9 (0.2)ab	1.9 (0.5)ab	1.7 (0.1)b	2.1 (0.2)a	1.6 (0.3)b	2.0 (0.5)ab	2.1 (0.3)ab
N/P	13.9 (1.2)a	13.1 (1.3)a	13.2 (1.0)a	12.7 (0.6)a	13.1 (0.8)a	14.0 (2.8)a	13.0 (1.1)a	13.3 (1.5)a
SLA	53 (8)ab	55 (8)ab	59 (8)ab	59 (8)a	47 (5)b	56 (7)ab	56 (13)ab	48 (5)ab
44 MAP (November 2005)								
N	14.6 (1.2)abc	14.8 (1.5)ab	16.2 (1.5)a	13.7 (2.4)bc	13.9	15.6 (0.8)ab	12.5 (2.1)c	13.3 (1.2)bc
P	1.1 (0.1)a	1.0 (0.1)a	1.2 (0.2)a	1.1 (0.1)a	1.1 (0.2)a	1.2 (0.2)a	1.0 (0.2)a	1.1 (0.2)a
Ca	9.7 (2.2)a	8.7 (1.4)a	9.4 (1.8)a	9.6 (3.4)a	8.5 (1.0)a	8.6 (2.1)a	9.3 (2.8)a	9.2 (2.5)a
K	4.8 (0.6)a	4.8 (0.6)a	5.1 (0.6)a	4.9 (0.7)a	4.8 (0.6)a	5.1 (0.7)a	4.7 (0.7)a	4.8 (0.7)a
Mg	2.1 (0.4)a	1.6 (0.3)a	2.1 (0.2)a	1.9 (0.5)a	1.9 (0.5)a	1.9 (0.2)a	2.2 (0.5)a	2.1 (0.2)a

Table 5 continued

	Treatments							
	I	IF	IL	R	RF	RL	S	SF
N/P	13.3 (1.5)a	14.2 (1.5)a	13.6 (1.6)a	12.7 (1.7)a	12.4 (1.8)a	13.3 (1.7)a	12.8 (1.0)a	12.7 (2.1)a
SLA	45 (3)a	46 (4)a	46 (5)a	48 (6)a	44 (4)a	44 (5)a	48 (10)a	47 (7)a
50 MAP (May 2006)								
N	13.7 (0.8)ab	14.6 (1.2)ab	16.1 (2.0)a	13.3 (0.9)b	14.3 (3.0)ab	14.7 (2.0)ab	12.9 (1.6)b	12.8 (1.1)b
P	0.8 (0.1)a	0.9 (0.1)a	1.1 (0.3)a	0.8 (0.1)a	1.0 (0.3)a	1.0 (0.2)a	0.8 (0.2)a	0.8 (0.1)a
Ca	10.7 (3.8)a	11.7 (1.7)a	8.6 (2.9)a	8.9 (2.6)a	10.9 (2.5)a	8.6 (3.4)a	11.9 (2.4)a	13.1 (3.0)a
K	5.0 (1.5)a	5.6 (1.8)a	6.6 (2.0)a	4.9 (0.9)a	5.4 (1.4)a	6.0 (1.8)a	4.9 (1.8)a	4.5 (0.7)a
Mg	1.8 (0.6)ab	1.6 (0.3)ab	1.5 (0.3)ab	1.8 (0.4)ab	1.9 (0.5)ab	1.4 (0.3)b	2.1 (0.4)a	2.0 (0.2)ab
N/P	16.6 (2.0)a	16.9 (1.4)a	15.4 (2.1)a	16.0 (2.7)a	15.2 (1.9)a	15.1 (2.0)a	15.7 (2.3)a	16.3 (2.9)a
SLA	42 (4)a	47 (11)a	44 (7)a	48 (11)a	47 (10)a	49 (13)a	41 (5)a	45 (8)a

See Table 3 for treatment codes. Values are mean and standard deviation (between brackets). Different letters in the same line mean significant differences ($P < 0.05$) between treatments by the Tukey test

* Before the first fertiliser application (March 2003)

between treatments decreased with time, as at 7 MAP tree heights in the I treatment were 1.30 times taller than in the R and S, but at the end of the study the ratio was only 1.12 and 1.16, respectively. These results contradict those reported by Soares et al. (2002) and Gómez-Rey et al. (2008) for lysimeter experiments carried out in controlled conditions (in which all soil horizons were disturbed) and in the absence of natural vegetation. In these experiments, trees of treatment S showed an early growth faster than in the I and R. In the present study, soil (texture loam to clay loam) disturbance by harrowing in the I treatment might have decreased bulk density and alleviated compaction (as reported by Madeira et al. 1989), which may have favoured the root system expansion during the early tree growth phase, and therefore the use of soil water and nutrient resources. This is in agreement with Magalhães (2000) in a previous experiment, in which tree growth response to incorporation of harvest residues in soils with finer texture and higher bulk density was faster than in soils with lighter texture and lower bulk density. Moreover, low amounts of understory vegetation in the I treatment (see Table 4) might also promote tree growth relatively to the R and S, as *Eucalyptus* growth suppression has been reported to be dependent on the competitive vegetation (Little et al. 2007).

The intercropped *Lupinus* negatively affected the early tree growth either when the harvest residues were incorporated (IL) or removed (RL). The suppression of tree growth observed in these treatments agrees with results found in other studies where leguminous cover reduced tree growth (Malik et al. 2001; Mendham et al. 2004). This may be attributed to competition between trees and understory vegetation for water resources, as *Eucalyptus* root mass, at early growth stage, is mostly found in the 30-cm top soil layers (Jones et al. 1999) and therefore may be more sensitive to summer drought in Mediterranean conditions. Thus, tree growth reduction observed in the first year in the IL and RL seems to be associated with understory biomass (7.5 and 2.1 times greater, respectively, than in the I and R treatments; see Table 4). The negative effect of *Lupinus* decreased with time, which may be associated with reduced competition between trees and the cover crop, as tree roots may have explored deeper layers (beyond 1 m soil depth; Fabião et al. 1987) of the soil profile; also, differences regarding understory biomass between treatments were smaller (see Table 4). At the end of the study, the positive effect in the RL relatively to the R treatment may be also ascribed to inputs of N through leguminous and P due to superphosphate application.

Despite great amounts of nutrients in the harvest residues, the removal of harvest residues may not have deleterious effects on tree nutritional status, when compared with their incorporation or maintenance on soil surface. This trend confirms which was observed in another field experiment (Jones et al. 1999; Magalhães 2000) and in a lysimetric experiment (Soares et al. 2002) where either the removal or the placement (incorporated or on the soil surface) of forest floor litter and harvest residues of the previous eucalypt plantation did not lead to significant differences in tree nutrient uptake. Also, soil net N mineralisation was not significantly affected by these treatments both in the field and lysimetric experiments (Azevedo 2000). Our results suggest that nutrients released from retained harvest residues were insufficient to enhance tree growth.

The lack of tree nutrition response to the harvest residue management is also supported by the fact that tree growth was enhanced (31, 18 and 40%, respectively, in I, R and S treatments) through repeated N fertiliser application, either in the absence or in the presence of harvest residues,

indicating that soil mineralisation rates were insufficient to meet N uptake for optimum tree growth. The tree growth response to N fertiliser application corroborates results obtained in young eucalypt plantations in sandy soils (close to the experimental site) and in the absence of harvest and other organic residues (Pereira et al. 1996), where optimum fertiliser addition (after 5 years) increased productivity by 19%. The maintenance of harvest residues in the site may enhance effects of fertiliser application, as tree growth increment was stronger in the I (31%) and S (40%) than in the R (18%) treatments. Differences between treatments seem to be related to nutrition of microorganisms, N-limited rather than C-limited especially in treatments with decomposing harvest residues (with high C/N ratio; see Table 1), as the soil available N may not be sufficient to fulfil both tree and organisms needs. Therefore, in sites with low natural fertility, independently of the harvest residue management, there will be the need to augment soil N supply through fertilisation if optimum tree growth rate is to be achieved.

Although fertilisation did not promote significant differences in understory biomass, greater biomass in fertilised than in unfertilised treatments by the end of the experiment suggests that fertilisation might have enhanced understory biomass accumulation. This may contribute to extend fertiliser effects by nutrient cycling and soil carbon accumulation. Understory biomass seemed to have stabilised in all treatments between 3 and 4 years after planting, as reported for similar eucalypt plantations in the same area by Fabião et al. (2002) and Carneiro et al. (2009).

Foliar analysis showed nutrient contents similar to those reported for several *E. globulus* plantations in central Portugal (Pereira et al. 1996) and southern Spain (González-Esparcia et al. 1985), but contents of P, Ca and Mg were greater than those measured by Merino et al. (2003) for wet areas of northwest Spain. One year after the last N fertiliser application, N/P ratio among treatments was slightly higher (15.1–16.9) than the optimum value (13–15) obtained by Cromer (1996) and Prado and Toro (1996) for eucalypt plantations, but much lower than that found by Merino et al. (2003), in sites (NW Spain) where soil N contents were ten times greater than in the present study, but with similar available P contents.

Foliar N/K ratio (2.7–2.9) is lower than the reference value given by Prado and Toro (1996) for *E. globulus* (3.9). The K/P ratio (5.4–6.2) was higher than the average ratio (5.0) reported by Judd et al. (1996) for young plantations in south-eastern Australia. These results suggest that small differences occur among treatments regarding nutrition, which is corroborated by the fact that linear correlations between N leaf content and tree growth, represented by its height ($R^2 = 0.57$, $P = 0.029$), were only found at 26 MAP (after the second fertiliser application). Also, results suggest that nutrition of P can be improved.

Three months after the first fertiliser application, N/Ca and N/Mg ratios were statistically higher (4.1 and 17.3, respectively) in the IF, RF and SF treatments than in the other treatments (1.9 and 8.7, respectively), suggesting that cation competition or antagonism (Mengel and Kirkby 2001) may explain these results and play a major role in the uptake: increasing N supply in the soil competitively depressed the uptake of Ca and Mg. This trend was still observed 8 months after the first fertiliser application, being the leaf Ca content in the fertilised treatments (mean of 4.8) significantly lower than in the others (5.2–6.4). Soil acidity and nitrification restrictions, as observed by Madeira et al. (2004) in eucalypt plantations in the neighbourhood, may favour high contents of NH_4^+ in soil and, therefore, the enhancement of the aforementioned antagonism. However, this may not negatively affect the tree nutritional status as eucalypt species assimilate NH_4^+ in preference to NO_3^- (Adams and Attiwill 1982; Garnett et al. 2001).

A strong decrease was observed in SLA with tree age. According to Fabião (1986), this pattern is associated with different leaf type (juvenile, intermediate and adult), the latter becoming narrower and thicker. Similar change was reported by England and Attiwill (2008) during leaf expansion of *Eucalyptus regnans*. Also, as leaves evolved from juvenile to adult, concentrations of N, P and K decreased while Ca concentration increased, which agrees with results reported by Azevedo (2000), for the region of the present study, and England and Attiwill (2008) for expanding *E. regnans* leaves. According to England and Attiwill (2008), this decrease may be the consequence of the nutrient concentration rates being much lower than the rate of dry matter accumulation, resulting in the nutrient dilution. However, the opposite was found relatively to leaf Ca concentration, which may be due to the accumulation of Ca in the cell-wall leaf components as Ca-pectate bonds (England and Attiwill, 2008). Our results also suggest that leaf evolving may change nutrient ratios, as N/P decreases from the juvenile (11.4–13.0) to the adult (13.1–15.9) stage.

Conclusions

The maintenance of harvest residues on the soil surface showed similar effects to their removal regarding tree nutrition and growth. Their incorporation into the soil also enhanced growth, but differences between treatments decreased along the study period. The intercropped leguminous plants increased the initial foliage N content, but depressed the early tree growth; however, at the end of the

study, leaf N contents were higher in the leguminous treatments than in the others, but growth was similar to treatments in which *Lupinus* was absent. Although harvest residue management led to similar tree nutritional status, application of N fertiliser, in the short term, promoted significantly higher levels of leaf N, independently of harvest residues being removed or maintained on the soil. Fertiliser application increased tree growth, especially when the residues were maintained on the soil surface. Under Mediterranean conditions, harvest residue incorporation into the soil associated with repeated N fertilisation may be the best management system to increase forest plantation productivity.

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